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Production and characterization of monoclonal antibodies reactive with the chicken interleukin-15 receptor alpha chain

Guangxing Li, Hyun S. Lillehoj*, Wongi Min

Parasite Biology, Epidemiology, and Systematics Laboratory, Animal and Natural Resource Institute, USDA-ARS, Beltsville, MD 20705, USA

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Abstract

We recently cloned the genes encoding chicken IL-15 and IL-15 receptor (R) α proteins. In this study, 12 monoclonal antibodies (mAbs) against recombinant chicken IL-15Rα were produced and characterized. By enzyme-linked immunosorbent assay (ELISA), all mAbs showed binding specificity for IL-15Rα, but not IL-2 or interferon-γ, and identified a 25.0 kDa protein by immunoblot analysis. Flow cytometric analysis revealed negligible expression of IL-15Rα on non-activated lymphocytes from the spleen, thymus or bursa, low but detectable expression on macrophages and high expression on concanavalin A-activated spleen lymphoblasts. Established chicken T cell (RP13) and macrophage (HD11) cell lines expressed substantially higher levels of IL-15Rα compared with a B cell line (RP9). Two mAbs inhibited IL-15 dependent proliferation of T cells suggesting that the tertiary structure of the protein domain of native IL-15Rα that binds to IL-15 is preserved in the recombinant receptor molecule. These mAbs will be useful reagents for further in vitro and in vivo studies of the biological functions of chicken IL-15 and its receptor. Published by Elsevier Science B.V.

Keywords: IL-15 receptor; Monoclonal antibody; Chickens

Abbreviations: BSA, bovine serum albumin; CMF-HBSS, calcium- and magnesium-free Hanks' balanced salt solution; con, concanavalin; ELISA, enzyme-linked immunosorbent assay; FCA, flow cytometric analysis; FCS, fetal calf serum; HRP, horseradish peroxidase; IFN, interferon; IL, interleukin; IMDM, Iscove's modified Dulbecco's medium; mAb, monoclonal antibody; MBP, maltose binding protein; NK, natural killer; PBS, phosphate buffered saline; PBS-T, PBS containing 0.05% Tween-20; PCR, polymerase chain reaction; R, receptor; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

* Corresponding author: Tel.: +1-301-504-8771; fax: +1-301-504-5103.
E-mail address: hlilleho@anri.barc.usda.gov (H.S. Lillehoj).

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1. Introduction

IL-15 is a cytokine with diverse biological manifestations, many of which are shared with IL-2 (Tagaya and Waldmann, 1999). Among these are (1) induction of the differentiation, proliferation and activation of T cells (Bamford et al., 1994; Burton et al., 1994; Grabstein et al., 1994; Armitage et al., 1995; Lodolce et al., 1998; Zhang et al., 1998), (2) chemoattraction of T cells and homing of circulating lymphocytes to peripheral lymph nodes (Wilkinson and Liew, 1995; McInnes et al., 1996; Lodolce et al., 1998; Oppenheimer-Marks et al., 1998), (3) protection against lymphocyte apoptosis (Lodolce et al., 1998; Vella et al., 1998), (4) facilitation of the growth and differentiation of activated B cells (Armitage et al., 1995), (5) promotion of the differentiation, maturation, activation and survival of natural killer (NK) cells, (6) costimulation with IL-12 to induce NK cell production of interferon (IFN)-γ and tumor necrosis factor (Carson et al., 1994, 1997; Mrozek et al., 1996; Lodolce et al., 1998), (7) development and maintenance of intestinal intraepithelial lymphocytes, particularly those bearing the T cell receptor $\gamma\delta$ complex (Lodolce et al., 1998; Kennedy et al., 2000) and (8) involvement in protective immune responses, allograft rejection and autoimmune diseases (Munger et al., 1995; Nishimura et al., 1996; Khan and Kasper, 1996; McInnes et al., 1996; Chehimi et al., 1997).

IL-15 exerts its biological functions following binding to a heterotrimeric cell surface receptor consisting of the IL-15Rα, IL-2/15Rβ and common receptor γ (γ_c) chains. The IL-2/15Rβ and γ_c polypeptides are shared with other interleukin receptors, IL-2/15Rβ with IL-2 (Bamford et al., 1994) and γ_c with IL-2, IL-4, IL-7 and IL-9 (Giri et al., 1994; Grabstein et al., 1994; De Jong et al., 1996). The IL-15Rα chain is ligand-specific, although it is structurally related to IL-2Rα (Giri et al., 1995; Kennedy and Linda, 1996). At the molecular level, IL-15Rα and IL-2Rα are single-pass transmembrane glycoproteins containing N- and O-linked glycosyl units with apparent molecular weights of 59–62 kDa (Dubois et al., 1999). Both contain a repeated extracellular binding motif alternatively known as the sushi domain, the glycoprotein-I motif or the short consensus repeat (Anderson et al., 1995; Giri et al., 1995). Physiologically, *IL-15R*α gene transcripts, unlike those of *IL-2R*α, are expressed by a variety of cell types and tissues. The ubiquitous expression of IL-15Rα is one of several mechanisms underlying the pleiotropic nature of the biological activities of IL-15 (Waldmann et al., 1998).

Little information is available concerning the biochemical or cellular properties of IL-15 or its receptor in nonmammalian species. Recently, we cloned the chicken *IL-15* (Lillehoj et al., 2001) genes from an expressed sequence tag library prepared from con A-activated spleen cells. We observed that chickens injected with the *IL-15* gene showed enhanced NK-cell activity and increased CD3 T lymphocytes suggesting a potential immunotherapeutic utility of chicken IL-15. However, the physiological basis underlying this effect is unknown. In order to understand better the molecular mechanism(s) by which IL-15 augments innate immunity, we developed a panel of mAbs against the chicken IL-15Rα chain and used them to characterize its molecular and cellular properties.

2. Materials and methods

2.1. Expression and cloning of the IL-15Rα gene

The coding region of the chicken *IL-15Rα* gene was cloned by polymerase chain reaction (PCR) from the expressed sequence tag, pat.pk0012.h3 (Tirunagaru et al., 2000) using the following primers (*Eco*R I and *Xba*I restriction sites are underlined):

sense primer, 5′-GACTGAATTCGACACGGCGCTGGTCAACACC-3′,

antisense primer, 5′-GTCATCTAGATCAGCCCGTGGGGATGTCACC-3′.

The PCR product was digested with *Eco*R I and *Xba*I and inserted into the corresponding sites of the pMAL-c2 expression vector (New England Biolabs, Beverly, MA) to form a sequence encoding a fusion protein of chicken IL-15Rα and maltose binding protein (IL-15Rα-MBP). The recombinant plasmid was transformed into competent thioredoxin reductase deficient *E. coli* AD494 (DE3) and transformants were selected on ampicillin plates. *E. coli* colonies harboring recombinant plasmid were grown at 37°C to $OD_{600} = 0.5$, induced with 1.0 mM isopropyl-β-D-thiogalactopyranoside for 3 h at 37°C and disrupted by sonication on ice (Misonix, Farmingdale, NY). The IL-15Rα-MBP fusion protein was purified on an amylose affinity column (New England Biolabs) according to the manufacturer's instructions and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions as described (Laemmli, 1970).

2.2. IL-15Rα mAbs

According to the procedure of Yun et al. (2000), BALB/c mice (National Cancer Institute, Frederick, MD) were immunized subcutaneously with 50 μg of IL-15Rα-MBP in Freund's complete adjuvant (Sigma, St. Louis, MO) and boosted weekly three times with an equivalent dose in Freund's incomplete adjuvant (Sigma). Sera were tested for IL-15Rα reactive antibodies enzyme-linked immunosorbent assay (ELISA) and mice containing high antibody titers were injected intravenously and intraperitonally with 10 μg of IL-15Rα-MBP in phosphate buffer saline (PBS). After 3 days, spleen lymphocytes were fused with SP2/0 myeloma cells (American Type Culture Collection, Manassas, VA) using 50% polyethylene glycol (Sigma). Hybridoma cells were cultured in Iscove's modified Dulbecco's medium (IMDM, Life Technologies, Gaithersburg, MD) containing 10% fetal calf serum (FCS, HyClone Laboratories, Logan, UT), hypoxanthine, aminopterin and thymidine (Sigma) and cloned twice by limiting dilution. Ascites fluids were produced in BALB/c mice following intraperitoneal injection of 2,6,14,20-tetramethylpentadecane (Sigma) and 5×10^6 hybridoma cells, clarified by centrifugation at $1000 \times g$ for 20 min, delipidated with PHML Liposorb (Calbiochem, La Jolla, CA) and stored at −20°C until use. Immunoglobulin isotypes were determined by a commercial kit (Life Technologies). Ascites immunoglobulins were isolated by FPLC (Amersham Pharmacia, Piscataway, NJ) in 1.0 ml aliquots diluted 10-fold in PBS, pH 7.2 on a 5.0 ml HiTrap protein G affinity column. After washing the column with application buffer, immunoglobulins were eluted with 0.1 M glycine, pH 2.8, neutralized with 1.0 M Tris–HCl, pH 9.0 and dialyzed

extensively against PBS. Protein concentrations were determined by a commercial kit (Sigma).

2.3. IL-15Rα ELISA

A total of 96 well flat-bottomed plates (Costar, Corning, NY) were coated overnight at 4°C with 100 μl of IL-15Rα-MBP at 10 μg/ml in 0.1 M bicarbonate-carbonate buffer, pH 9.6, washed three times with PBS, pH 7.2 containing 0.05% Tween-20 (PBS-T), blocked with 200 μl of PBS containing 1% bovine serum albumin (BSA) for 1 h at room temperature and washed three times with PBS-T. About 100 μl of hybridoma supernatants were added, incubated for 2 h at room temperature, wells washed three times with PBS-T, 100 μl of appropriately diluted horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (H + L, Sigma) added, incubated for 1 h at room temperature, washed three times, enzyme activity detected with 100 μl of 0.01% 3,3′5,5′-tetramethylbenzidine (Sigma) in 0.05 M phosphate-citrate buffer, pH 5.0 for 15 min and stopped with 50 μl of 2 M H_2SO_4. Absorbency at 450 nm was measured with a microplate reader (BioRad, Richmond, CA).

2.4. Immunoblot analysis

IL-15Rα-MBP was mixed with SDS-PAGE sample buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue), heated at 94°C for 4 min, applied to a 15% SDS-polyacrylamide gel and electroblotted to nitrocellulose (Millipore, Bedford, MA) as described (Laemmli, 1970; Towbin et al., 1979). The membrane was blocked in PBS containing 1% non-fat dry milk at 4°C overnight, incubated with hybridoma supernatants for 1 h at room temperature, washed with PBS containing 1% BSA, incubated with HRP-conjugated goat anti-mouse IgG antibody (H + L) for 30 min at room temperature, washed five times with PBS and five times with distilled water and developed using Sigma Fast DAB peroxidase substrate (Sigma).

2.5. Inhibition of IL-15-stimulated cell proliferation

Plasmids encoding chicken recombinant IL-15 (pcDNA3-chIL-15) and IL-2 (pcDNA3-chIL-2) were transfected into COS-7 and CHO-K1 cells, respectively using Lipofectamine (Life Technologies) as described (Choi et al., 1999). After culture in IMDM containing 10% FCS for 3 days, supernatants were collected, centrifuged at 5000 × *g* for 10 min and used as sources of recombinant IL-15 and IL-2. Con A activation of spleen cells was performed as described (Myers et al., 1992; Choi et al., 1999). Briefly, spleens from 5-week-old SPAFAS chickens were pressed gently through stainless steel screens into a Petri dish containing calcium- and magnesium-free Hanks' balanced salt solution (CMF-HBSS, Sigma), cells purified by centrifugation through Histopaque-1077 (Sigma), washed three times with CMF-HBSS and resuspended in IMDM containing 10% FCS, 1.0 mM sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, 0.1 mM nonessential amino acid, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, pH 7.3, 2.0 mM glutamine and 5 μg/ml 5-fluorocytosine (all from Sigma). Cells were seeded at 5×10^6/ml in six well flat

bottomed culture plates, incubated with 12.5 μg/ml con A (Amersham Pharmacia) at 41°C and 5% CO_2 for 48 h, dead cells removed by centrifugation through Histopaque-1077, and treated with 0.05 M α-methyl-mannoside to inactivate con A. About 100 μl of affinity purified mAb immunoglobulins serially diluted to 0.32, 0.63, 1.25, 2.5 and10 μg/ml were incubated with 100 μl of activated spleen cells at 41°C for 2 h, 50 μl of IL-15 or IL-2 added to each well and the cells incubated at 41°C for 48 h. Proliferation was measured by the WST-8 assay using the Cell Counting Kit 8 (Dojindo Molecular Technologies, Gaithersburg, MD).

2.6. Flow cytometric analysis (FCA)

Single cell suspensions of spleen, thymus, bursa, and peripheral blood leukocyte-derived macrophages (PBLMs), prepared as described above, and the RP9, RP13 and HD11 cell lines were resuspended in HBSS without phenol red supplemented with 3% FCS and 0.01% sodium azide (FCA buffer), incubated on ice for 30 min with hybridoma supernatants diluted in FCA buffer, washed three times and resuspended in 50 μl of FCA buffer (Lillehoj et al., 1988). About 50 μl of fluorescein isothiocyanate-labeled rabbit anti-mouse IgG antibody (Sigma) was added, incubated for 30 min on ice and fluorescence measured in an EPICS XL-MCL flow cytometer (Coulter, Miami, FL) with 10,000 viable cells. Mouse mAbs used in indirect immunofluorescence staining were 1–9B (anti-CD3) (Lillehoj et al., 1993), CTLA 4 (anti-CD4) (Lillehoj et al., 1988), CTLA 8 (anti-CD8) (Lillehoj et al., 1988), Macrophage (K1) (Kaspers et al., 1993), αβ T cell receptor (TCR2) (Chen et al., 1986), γδ T cell receptor (TCR1) (Chen et al., 1986) and Pan lymphocytes (CD45) (Chen and Cooper, 1987).

2.7. Statistical analysis

Data were analyzed by ANOVA with MINITAB software version 10.1 (Minitab, State College, PA) and are presented as mean values ± standard deviations.

3. Results

3.1. Production of IL-15Rα mAbs

From three separate cell fusions, twelve hybridomas were cloned and selected for further study based upon ELISA reactivity with IL-15Rα-MBP and nonreactivity with IL-2, IFN-γ and MBP (Table 1). Seven mAbs were IgG1-κ, two IgG3-κ, and one each IgG2a-κ, IgG2b-κ and IgM-κ. A representative ELISA standard curve using purified immunoglobulin from hybridoma 5.3.1 is shown in Fig. 1.

3.2. Immunoblot analysis

Fig. 2 illustrates immunoblot analysis of IL-15α-MBP before and after treatment with Factor Xa using mAb 5.3.1. An immunoreactive band with an apparent molecular weight

Table 1
ELISA and immunoblot reactivities of IL-15Rα monoclonal antibodies

Description	Isotype	ELISA[a]				Immunoblot[b]	Neutralization
		IL-15Rα	MBP	IL-2	IFN-γ		
2.19.1	IgG1-κ	+	−	−	−	+	−
5.3.1	IgG3-κ	+	−	−	−	+	+
4.17.1	IgG3-κ	+	−	−	−	+	−
1.5.1	IgG1-κ	+	−	−	−	+	−
2.16.2	IgG1-κ	+	−	−	−	+	−
3.12.1	IgG1-κ	+	−	−	−	+	−
1.22.1	IgG1-κ	+	−	−	−	+	−
2.9.1	IgG1-κ	+	−	−	−	+	+
2.17.2	IgG2b-κ	+	−	−	−	+	−
1.16.2	IgM-κ	+	−	−	−	+	−
2.15.2	IgG2a-κ	+	−	−	−	+	−
1.12.1	IgG1-κ	+	−	−	−	+	−

[a] ELISA microplate wells were coated with IL-15Rα-MBP, MBP, IL-2-MBP or IFN-γ-MBP and sequentially incubated with hybridoma supernatants. Optical density values greater than twice the value of the negative control (wells coated with 1% BSA alone) were considered reactive (+). Optical density values less than or equal to twice the value of the negative control were considered non-reactive (−).

[b] All mAbs reacted with IL-15Rα-MBP, but MBP and IFN-α-MBP.

of approximately 68.0 kDa was observed using untreated IL-15-MBP (lane 2). Following Factor Xa treatment to separate the fusion partners, a 25.0 kDa polypeptide was noted (lane 1). The smaller protein at approximately 18.0 kDa most likely represents a proteolytic fragment of IL-15α. MBP and IFN-γ-MBP fusion protein were non-reactive with mAb 5.3.1 (lanes 3 and 4). Identical results were seen using the remaining 11 mAbs.

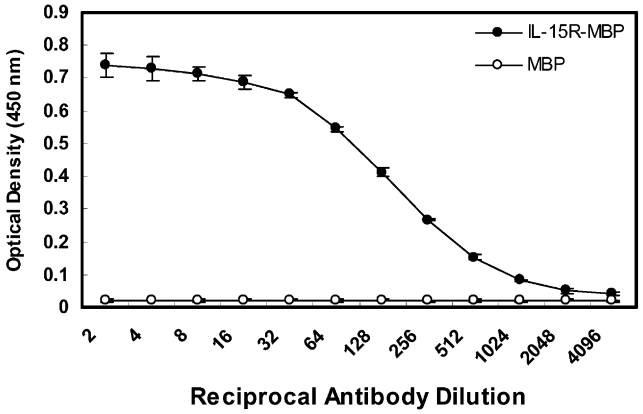

Fig. 1. ELISA reactivity of IL-15Rα mAbs with IL-15Rα-MBP. Microtiter plates were coated with serial dilutions of IL-15Rα-MBP (solid symbol) or MBP (open symbol) and sequentially incubated with mAb 5.3.1, HRP-anti-mouse IgG antibody (H + L) and TMB substrate. Data are presented as mean optical density values from triplicate samples ± S.D.

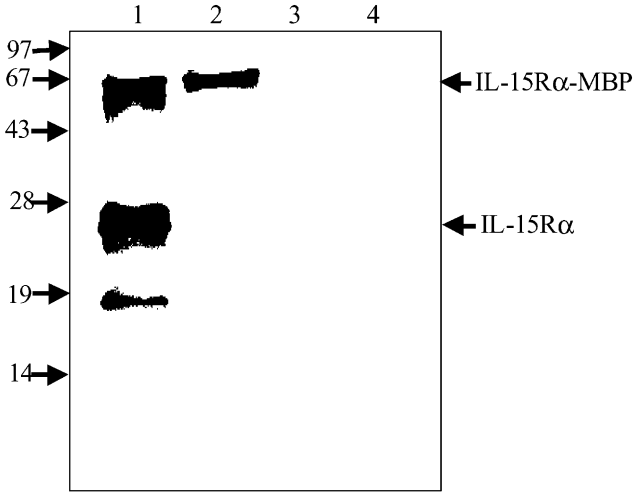

Fig. 2. Immunoblot analysis of IL-15Rα using mAb5.3.1. Lane 1, recombinant IL-15Rα-MBP following treatment with Factor Xa. Lane 2, IL-15Rα-MBP prior to Factor Xa treatment. Lane 3, MBP. Lane 4, IFN-γ-MBP fusion protein. The relative mobility (Mr) of prestained protein size markers is indicated on the left in kDa. The positions of the 68.0 kDa IL-15Rα-MBP protein and 25.0 kDa IL-15Rα protein are indicated on the left.

Furthermore, IL-15Rα mAb immunoblot analysis of detergent lysates from normal thymus and spleen cells and con A-activated spleen cells revealed a faint but reproducible immunoreactive band in the 50–60 kDa region of the gel (data not shown). The larger size of this band compared with that of the recombinant IL-15Rα is expected due to glycosylation of the native receptor molecule (Dubois et al., 1999).

Table 2
Flow cytometric analysis of IL-15Rα mAbs

Tissue	Percent staining (%)[a]				
	5.3.1	2.19.1	2.9.1	2.17.2	1.16.2
Thymus	1.62	0.87	0.88	1.35	1.44
Bursa	2.93	0.91	1.23	1.36	2.28
PBLMs	5.35	4.20	4.74	2.54	6.61
Spleen	1.12	1.33	1.94	2.18	2.37
24 h con A[b]	12.1	8.64	12.6	9.88	16.4
48 h con A[b]	18.8	15.6	19.9	16.8	17.4
RP9	11.6	6.08	4.17	1.78	1.81
RP13	13.8	9.74	6.52	11.6	40.2
HD11	11.1	8.64	8.71	5.29	6.69

[a] The single cell suspensions were stained with the supernatants of hybridoma cells and analyzed by as described in Section 2. Data are expressed as the percent (%) staining. Representative results from two independent experiments are shown.

[b] Spleen cells were activated with con A for 24 or 48 h.

3.3. Cell and tissue distribution of IL-15Rα

Mammalian IL-15Rα chains are ubiquitously expressed in normal cells and tissues (Giri et al., 1995) as well as mitogen-activated macrophages, NK cells and $CD4^+$ and $CD8^+$ T cells (Chae et al., 1996). To assess the distribution of chicken IL-15Rα, a variety of primary cells and established cell lines were analyzed by FCA. In general, negligible levels (<3%) of IL-15Rα were detected on non-activated cells, with the possible exception of PBLMs (3–7%) (Table 2). By contrast, con A-stimulated spleen cells demonstrated remarkably increased expression (9–20%). As a negative control, the mouse mAb which detect human T cells (13-3A1: ATCC, Rockville, MD) was used since it stains less than 2% of chicken cells. Representative staining profiles using mAb 1.16.2 are illustrated in Fig. 3. Staining with IL-15Rα mAbs indicated their order of reactivity as RP13 > HD11 > RP9 (Table 2).

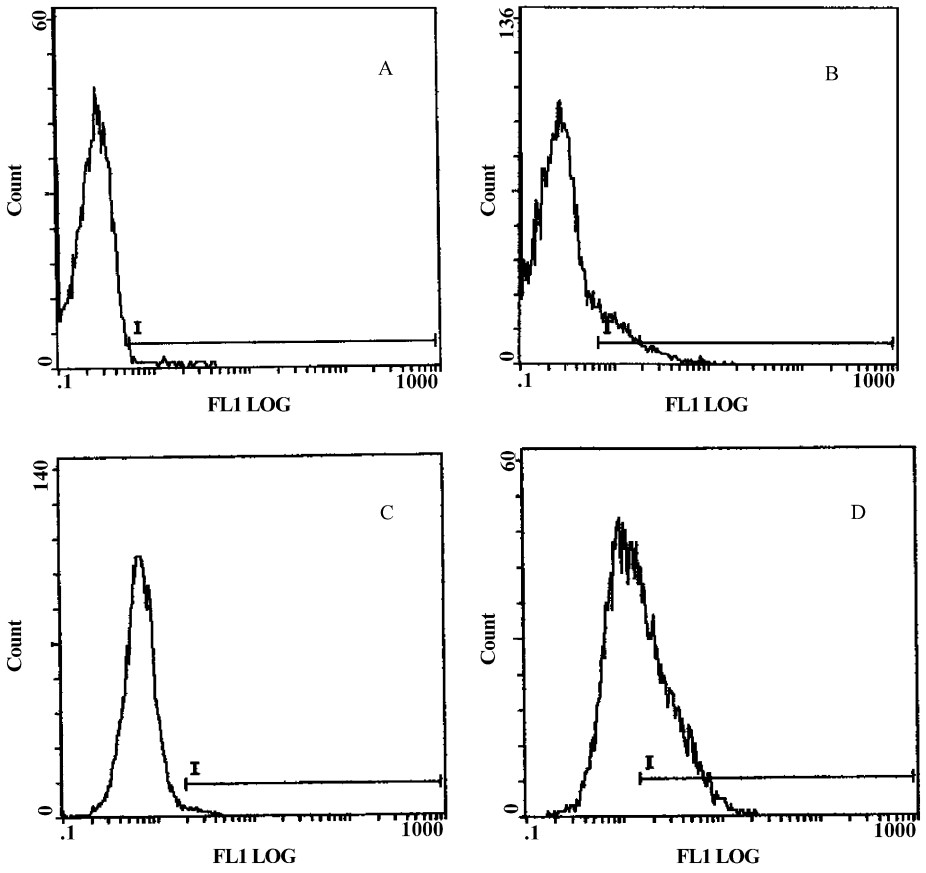

Fig. 3. Flow cytometric analysis of IL-15Rα: (A) non-activated spleen lymphocytes; (B) 48 h con A-stimulated spleen lymphoblasts; (C) RP9 cells; and (D) RP13 cells were stained with mAb 1.16.2 and analyzed by FCA as described in Section 2.

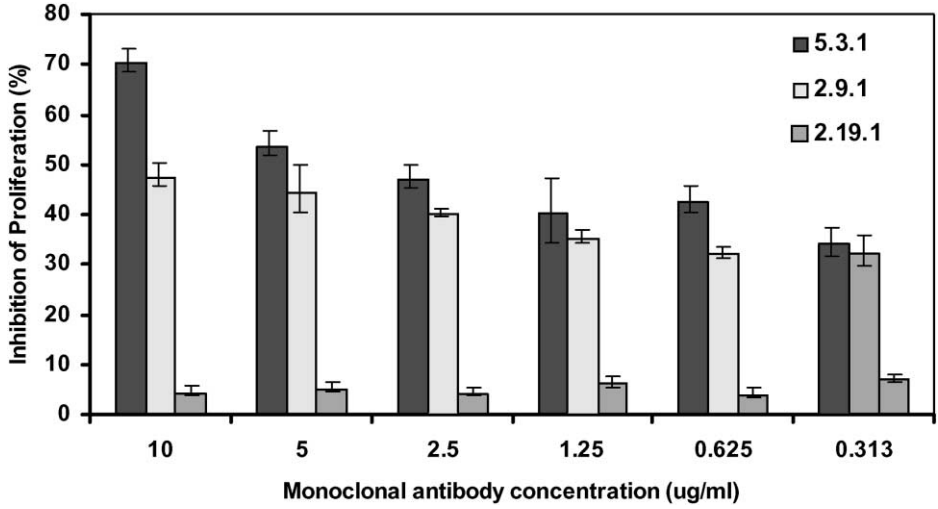

Fig. 4. Inhibition of IL-15-stimulated proliferation of conA-activated spleen lymphocytes. Con A-activated cells were incubated with IMDM media alone (negative control), IL-15 containing culture supernatant from COS 7 cells transfected with the pcDNA3-chIL-15 expression plasmid (Choi et al., 1999) (positive control) or varying concentrations of purified mAbs 5.3.1, 2.9.1, and 2.19.1 in the presence of IL-15 (sample). Cell proliferation was measured as described in Section 2 and the percent inhibition of proliferation was calculated by the following formula: percent inhibition = (positive control − sample)/(positive control − negative control) × 100.

3.4. Inhibition of IL-15-stimulated spleen cell proliferation

To investigate the biological activity of the mAbs which detect chicken IL-15 Rα, their ability to inhibit IL-15-driven T-cell proliferation was tested. As shown in Fig. 4, out of 12 mAbs tested, only the mAbs 5.3.1 and 2.9.1 inhibited IL-15-induced proliferation of con A-activated spleen cells in dose-dependent manners. Neither mAb blocked IL-2-stimulated proliferation (data not shown). By contrast, mAb 2.19.1 was incapable of inhibiting IL-15-induced proliferation at any of the concentrations tested. Control mouse mAb which detects human T cells (13-3A1: ATCC, Rockville, MD) did not show any inhibitory activity at the same concentration of mAB used. Representative staining profiles using mAb 1.16.2 are illustrated in Fig. 3. These results suggested that at least some of the mAbs we produced were reactive with protein domains of native IL-15Rα directly associated with, or closely adjacent to, the site at which IL-15 binds. By implication, we predict that the recombinant molecule used for immunization should possess the proper tertiary structural features of the native molecule necessary for bioactivity.

4. Discussion

In the present study, we established 12 mAbs against the chicken IL-15Rα chain and used them to characterize the biochemical and cellular properties of recombinant and native forms of the receptor. Our results demonstrated that (1) all mAbs were specificity

reactive with recombinant IL-15Rα both in ELISA and immunoblot assays, (2) all mAbs reacted with native IL-15Rα on the cell surface by FCA, (3) expression of native IL-15Rα on activated lymphocytes was substantially higher compared with nonactivated cells, (4) chicken T cell and macrophage lines expressed more IL-15Rα than a B cell line and (5) a subset of mAbs blocked IL-15-stimulated proliferation of spleen cells and thereby are suggested to have reacted with a protein domain of the receptor molecule involved in binding IL-15.

Compared with IL-2, the broader biological functions of IL-15 have been attributed to ubiquitous expression of the IL-15R complex. By Northern blot analysis, mammalian IL-15Rα transcripts were identified in lymphoid (T cells, B cells, macrophages, thymic and bone marrow stromal cell lines) as well as nonlymphoid (liver, heart, spleen, lung, skeletal muscle, activated vascular endothelial) cells (Giri et al., 1995). Quantitatively, however, many researchers found that expression of IL-15Rα on the cell surface was very low (∼1000 sites/cell) and most was localized intracellularly (Dubois et al., 1999). Chae et al. (1996) observed that normal macrophages, NK cells and T cells expressed low levels of IL-15Rα protein but its production was up-regulated following phytohemagglutinin activation. Other stimuli also enhanced receptor expression, such as IL-2 or CD3 antibody plus phorbol myristyl acetate treatment of T cells (Giri et al., 1994) and IFN-γ stimulation of macrophages (Giri et al., 1995). Our experimental results are consistent with these observations. While we noted very low levels of IL-15Rα chain expression on the surface of resting cells, there was a substantial increase in expression following stimulation by con A. Our results quantifying expression of IL-15Rα on chicken T cell and macrophage lines to a greater extent than a B cell line also substantiates prior observations in mammalian species (Giri et al., 1995).

Despite the fact that the mammalian IL-15Rα chains that have been characterized do not possess the function of signal transduction or efficient receptor internalization, this molecule nevertheless contributes to the formation of a specific high affinity cytokine receptor complex controlling the sensitivity of cells to physiological concentration of its cognate cytokine ligand (Giri et al., 1995; Lodolce et al., 1998). Kennedy et al. (2000) observed that IL-$15^{-/-}$ mice did not develop more severe immunodeficiency compared with IL-$15R\alpha^{-/-}$ mice thus suggesting that the trimeric high affinity IL-15R complex is required for normal physiological responses to IL-15. However, De Jong et al. (1998) demonstrated in humans a functional IL-15R with intermediate cytokine affinity composed of a heterodimeric IL-2/15Rβ and γ_c chain complex. Our failure to achieve absolute inhibition of IL-15-stimulated spleen cell proliferation may be that the antibody concentration was not enough to induce complete inhibition.

Several lines of evidence advocate the use of IL-15Rα antibodies, particularly those capable of blocking IL-15 activity, as immunotherapeutic agents. For example, IL-15 is known to mediate immune responses to viral (Chehimi et al., 1997), bacterial (Nishimura et al., 1996) and protozoan (Khan and Kasper, 1996) infections and is involved in tumor cell immunity (Munger et al., 1995). IL-15 plays a critical role in the pathogenesis of autoimmune diseases (McInnes et al., 1996) and administration of soluble IL-15Rα suppressed development of collagen-induced arthritis in an animal model (Ruchatz et al., 1998). We reported previously that DNA immunization with the pcDNA vector carrying chicken IL-15 lead to enhanced NK-cell activity and increased CD3 T lymphocytes likely

as a result of augmented production of this cytokine and a consequent immunoenhancing effect (Lillehoj et al., 2001). In theory, therefore, blocking the activity of this important immunoregulatory cytokine should have the effect of dampening the immune response and thereby provides a new perspective for treatment of inflammatory diseases. We are currently investigating the IL-15Rα mAbs described herein in this regard.

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